Capillary Electrophoresis and Fluorescence Excitation-Emission Matrix Spectroscopy for Characterization of Humic Substances

Zhongqi He*
USDA-ARS
New England Plant, Soil, and Water Lab.
Orono, ME 04469

Tsutomu Ohno
Dep. of Plant, Soil, and Environmental Sci.
Univ. of Maine
Orono, ME 04469

Fengchang Wu
Institute of Geochemistry
Chinese Academy of Sciences
Guiyang, China 550002

Daniel C. Olk
USDA-ARS
National Soil Tilth Lab
Ames, IA 50011

C. Wayne Honeycutt
Modesto Olanya
USDA-ARS
New England Plant, Soil, and Water Lab.
Orono, ME 04469

Capillary electrophoresis (CE) and fluorescence spectroscopy have been used in natural organic matter (NOM) studies. In this study, we characterized five fulvic acids (FAs), six humic acids (HAs), and two unprocessed NOM samples obtained from the International Humic Substances Society (IHSS) using these two analytical methods. The electropherograms of all samples revealed three peak features. The first and third peaks were sharp. The second peak had a broad, hump-shaped feature. The pattern and shapes of these peaks were different among the FA, HA, and unprocessed NOM samples. Excitation-emission matrix (EEM) fluorescence spectroscopic analysis revealed that each of the 13 investigated samples contained four components. However, the relative amounts of the four components varied with sample origin. Autoclaving these samples for 1 h (heat decomposition) produced additional CE peaks and changed portions of the four fluorophore components, indicating that both methods can be used to investigate the dynamics of NOM decomposition. Although four fluorophore components were present in each of the three CE fractions, their relative abundances varied among the three CE fractions. Specifically, Fraction 1 and 2 were rich in Component 1 and 4, but sparse in Component 2, compared with their original samples. Fraction 2 also contained less Component 3. The distribution of the four components in Fraction 3 was similar to that of the original samples. The mutual relevance of data collected from each of the two methods provided novel insight into the correlation of complex NOM fluorescence spectra to specific NOM fractions.

Abbreviations: CE, capillary electrophoresis; EEM, excitation-emission matrix; FA, fulvic acid; HA, humic acid; IHSS, International Humic Substances Society; NOM, natural organic matter; PARAFAC, parallel factor analysis.

Humic substances are ubiquitous natural materials present in large amounts in soils, sediments, and waters as a product of the chemical and biological transformations of animal and plant residues (Janos, 2003). They are important contributors to the global cycles of C, N, P, and S in the bio- and geospheres (Schmitt-Kopplin and Junkers, 2003). Because of their ability to interact with various components of the environment, humic substances play important roles in soil and aquatic chemical processes and therefore have attracted the attention of soil and environmental chemists (Schmitt et al., 1997; Xing, 2001; Janos, 2003; Ubner et al., 2004; Wang and Xing, 2005; He et al., 2006; Makarov and Malysheva, 2006; Muscolo et al., 2007). As humic substances are complex, heterogeneous, and polydisperse mixtures of non-stoichiometric composition, the investigation on their structure and function remains a significant challenge in modern analytical chemistry (Janos, 2003; Schmitt-Kopplin and Kettrup, 2003; Schmitt-Kopplin and Junkers, 2003; Jeong et al., 2007).

Capillary electrophoresis is a relatively new and growing analytic approach used in humic substance studies (Janos, 2003; Schmitt-Kopplin and Junkers, 2003; Peuravuori et al., 2004). Electrophoretic techniques are suitable for the separation and characterization of humic substances because of the polyelectrolytic nature of humic substances (Pompe et al., 1996; Schmitt-Kopplin and Kettrup, 2003; Ubner et al., 2004). The CE separation in uncoated capillaries is the result of the combination of an electroosmotic flow that is produced by an applied voltage and the fractionation by different charge-to-size ratios. Capillary electrophoresis features short analysis times, selectivity of analytes, and high resolution (Pompe et

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*Corresponding author (Zhongqi.He@ars.usda.gov).

al., 1996). In general, two or multiple peaks are observed on the eletropherograms of humic substances, dependent on their origin, charge, and size. Thus, CE has been used in a "finger-print" characterization of humic substances (Pompe et al., 1996; Egeberg and Bergli, 2002; Schmitt-Kopplin and Junkers, 2003). For example, the humic acids (HA) and fulvic acids (FA) from different sources exhibited characteristic, individual electropherograms when they were run with borate-phosphate buffer (3 m*M* KH_2PO_4, 6 m*M* $Na_2B_4O_7$, pH 8.9) (Pompe et al., 1996).

Excitation-emission matrix (EEM) fluorescence spectroscopy has been applied recently in studying humic substances (Alberts and Takacs, 2004; Wu et al., 2004; Sierra et al., 2005; He et al., 2006) and other NOM components (Chen et al., 2003; Cory and Mcknight, 2005; Ohno and Bro, 2006). This method measures emission spectra across a range of excitation wavelengths, resulting in a landscape surface defined by the fluorescence intensity at pairs of excitation and emission wavelengths (Merrit and Erich, 2003; Sierra et al., 2005; Ohno and Bro, 2006). As EEM fluorescence spectroscopy produces much more spectral information than the traditional fluorescence approaches, a multiway data analysis method, parallel factor analysis (PARAFAC), has been used to decompose the suite of EEM landscapes into chemically meaningful fluorophore components (Anderson and Bro, 2003; Bro and Kiers, 2003; Ohno and Bro, 2006). PARAFAC analysis has shown that eight distinct fluorescent fraction groups (four biogenic terrestrial, two anthropogenic, and two protein-like) can be identified in dissolved organic matter that originated from a temperate estuary and its catchment in Denmark (Stedmon and Markager, 2005). Hall et al. (2005) reported that three PARAFAC components best modeled their dissolved organic matter from the Mystic River watershed in Massachusetts.

As humic substances are more or less a random association of organic components, it seems not worthwhile to make extreme efforts to know exactly what the molecule is at present. On the other side, both CE and EEM fluorescence spectroscopy can resolve a more limited number of components (i.e., CE peaks and fluorescent fraction groups) from the random association. Thus, for modeling purposes, these components are very useful in describing how these humic substances in general react with soil surfaces or ions in soil solution (He et al., 2006; Ohno and Bro, 2006). However, to our knowledge, investigation of humic substances or other NOM using the two methods together has not been reported so far. Therefore, in this project, we characterized six HAs, five FAs, and two unprocessed NOM samples obtained from the IHSS using CE; and analyzed these IHSS samples using EEM spectroscopy with PARAFAC modeling to gain a better understanding of the make-up of these NOM samples.

Table 1. Elemental analysis of IHSS humic acid (HA), fulvic acid (FA), and natural organic matter (NOM) samples

Source†	Type	IHSS No.	C	H	O	N	S	P
					%			
Suwannee River	HA standard II	2S101H	52.63	4.40	42.04	1.17	0.54	0.013
Suwannee River	FA standard II	2S101F	52.34	4.36	42.98	0.67	0.46	0.004
Suwannee River	FA reference	1R101F	53.04	4.36	43.91	0.75	0.46	< 0.01
Suwannee River	NOM reference	1R101N	48.8	3.9	39.7	1.02	0.6	0.02
Nordic Reservoir	HA reference	1R105H	53.33	3.97	43.09	1.16	0.58	0.01
Nordic Reservoir	FA reference	1R105F	52.31	3.98	45.12	0.68	0.46	< 0.01
Nordic Reservoir	NOM reference	1R108N	53.2	5.7	-‡	1.1	–	–
Waskish peat	HA reference	1R107H	54.72	4.04	38.54	1.47	0.36	0.31
Waskish peat	FA reference	1R107F	53.63	4.24	41.81	1.07	0.29	0.12
Pahokee peat	HA reference	1R103H	56.84	3.60	36.62	3.74	0.70	0.03
Leonardite	HA standard	1S104H	63.81	3.70	31.27	1.23	0.76	< 0.01
Elliott soil	HA standard	1S102H	58.13	3.68	34.08	4.14	0.44	0.24
Elliott soil	FA standard II	2S102F	50.12	4.28	42.61	3.75	0.89	0.12

† Data are from the supplier's information.

‡ Data are not reported.

MATERIALS AND METHODS

Humic Substances

Thirteen humic substances (six HA, five FA, and two unprocessed NOM) were obtained from IHSS (Table 1). Seven were from aquatic sources, and six were from terrestrial sources. Details of the collection and processing methods are available at the IHSS website (www.ihss.gatech.edu, verified 9 June 2008). Stock solutions were made by weighing 10 to 20 mg of sample in a 2-mL micro tube and then adding appropriate volumes of 0.05 *M* NaOH to make a final concentration of 10 mg dry matter mL^{-1}. A portion (about 0.5 mL) of the stock solutions was autoclaved for 1 h at 121°C to examine the effect of heat decomposition on the CE and EEM fluorescence spectral characteristics of these samples. Each autoclaved stock solution was filtered through a 45-μm pore filter device to remove any particulate or coagulate materials produced during autoclaving.

Capillary Electrophoresis

The CE instrument was an Agilent capillary electrophoresis system (Agilent Technologies, Wilmington, DE). The running buffer was 3 m*M* KH_2PO_4–6 m*M* $Na_2B_4O_7$, pH 8.9 (referred to as borate buffer, hereinafter). The capillary column (fused silica, 56 cm length × 75 μm i.d.) was flushed for 3 min with the borate buffer before sample loading. The sample concentration for CE was 400 mg L^{-1} in 1.25 m*M* NaOH diluted from stock solutions with deionized water (Pompe et al., 1996). The sample was loaded into the column by pressure injection (500 Pa for 5 sec). The separation was performed at 30°C and a voltage of 30 kV with positive polarity. A voltage ramp was on for 0.1 min. The detection wavelength was set at 214 nm.

Collection of Capillary Electrophoresis Fractions

To collect sufficient quantities of CE fractions for EEM fluorescence measurements, the loading time of CE samples was extended from 5 s to 30 or 40 s, and the CE separation was repeated 10 to 24 times (Table 2). Each fraction was collected in a micro vial which was filled with 0.1 mL of borate buffer. The fraction was eluted into the micro vial by pressure mode.

Fluorescence Measurements

The samples for CE analysis were further diluted with borate buffer so that it would have an ultraviolet (UV) light absorbance at 240 nm of about 0.1. Fluorescence measurements were obtained using a Hitachi F-4500 spectrofluorometer. Instrumental parameters were excitation (EX) and emission (EM) slits, 5 nm; response time, 8 s; and scan speed 240 nm min^{-1}. The excitation-emission matrix

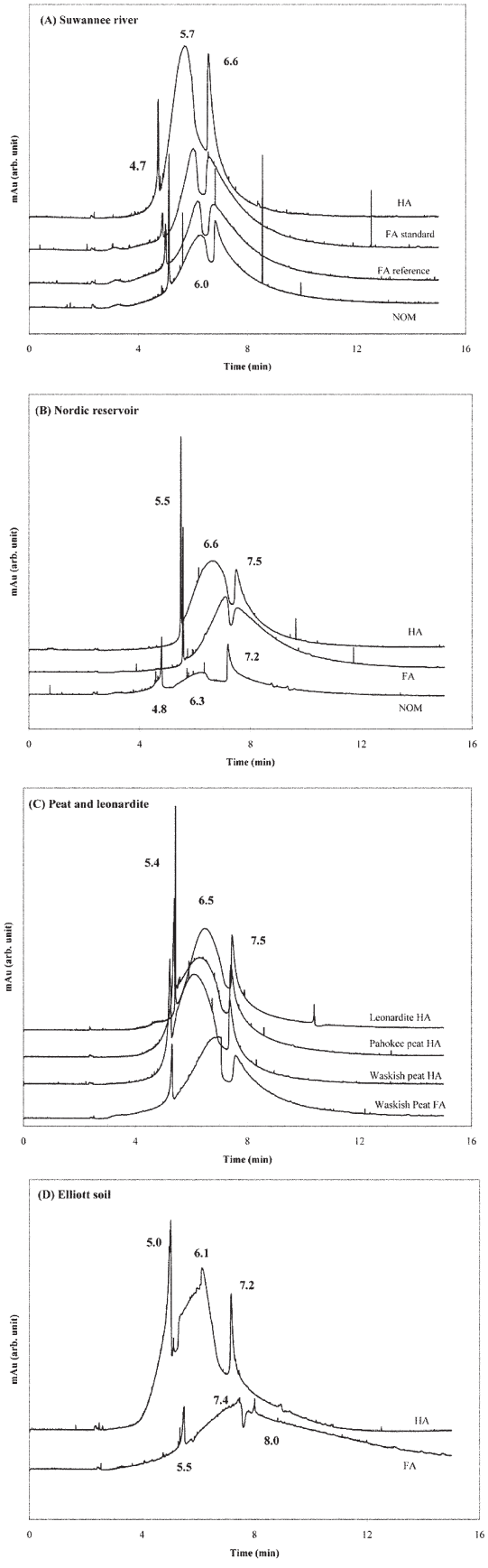

Fig. 1. Capillary electropherograms of the IHSS samples. HA, FA, and unprocessed NOM samples were from Suwannee River (A), Nordic reservoir (B), peat and leonardite sources (C), or Elliott soil (D).

Table 2. Concentration of three fractions separated by capillary electrophoresis.

Sample	Loading time	Number of sample collections	Collection width	Absorbance at 240 nm†		
				Peak 1	Peak 2	Peak 3
	s		%			
Suwannee HA	30	20	15	0.35	1.23	0.94
Suwannee FA std	30	24	15	0.15	0.42	0.34
Suwannee FA ref	30	14	10	0.17	0.23	0.37
Suwannee NOM	30	20	10	0.18	0.29	0.36
Nordic NOM	40	16	10	0.16	NC‡	0.25
Elliott soil HA	30	10	8	0.09	0.07	0.05
Waskish peat FA	40	23	10	0.05	0.12	0.12
Waskish peat HA	30	20	15	0.25	0.27	0.14
Pahokee peat HA	40	23	15	0.69	1.2	0.39
Leonardite HA Nordic	40	12	15	0.24	0.57	0.34
HA	40	20	15	0.25	0.84	0.69
Nordic FA	40	15	15	0.15	0.61	0.74
Elliott Soil FA	40	20	15	-§	–	–

† The volume of each fraction was 0.1 mL except Elliott soil HA which volume was 0.4 mL.

‡ The peak was too flat to be recognized and collected by the control program of the CE system.

§ Not recorded.

(EEM) fluorescence landscape was obtained by setting the EX range from 240 to 400 nm and EM range from 300 to 500 nm in 3-nm increments. Subtraction of a buffer blank EEM from each sample EEM was used to remove Raman scatter lines from the spectra.

The PARAFAC modeling was conducted with MATLAB version 7.0.4, Release 14 (Mathworks, Natick, MA) using PLS_Toolbox version 4.0 (Eigenvector Research, Manson, WA). A non-negativity constraint was applied to each dimension to allow only chemically relevant results because negative concentrations and fluorescence intensities are chemically impossible. The PARAFAC models with two to eight components were computed. The number of components in the data set was assessed by the core consistency diagnostic score which should be close to 100% for appropriate models.

RESULTS

Capillary Electrophoresis Profiles of IHSS Samples

Electropherograms of the 13 investigated samples were reproducible with respect to both the peak number and shapes (Fig. 1). The migration time of the peaks gradually shifted to longer times with repeated runs because of relative changes in the electroosmotic flow (i. e. different capillary surface conditions; Schmitt-Kopplin et al., 1998a). The focus of this work was more on the peak pattern and shape rather than the precise migration time parameter of each peak due to the fact that no internal standard was used in this study.

All four electropherograms of the samples derived from the Suwannee River contained three peaks (Fig. 1A). The first peak around 4.7 min was sharp, the second peak around 5.7 min was broad, and the third peak around 6.6 min was sharp but with a trailing character. All three peaks were superimposed on a broad background, which is frequently referred to as a humic "hump" (Garrison et al., 1995; Schmitt-Kopplin and Junkers, 2003). Other randomly appearing vertical lines (spikes) in the electropherograms were due to air bubbles in the buffer, aggregation of humic substances, and/or other artifacts which were of no relevance or interest (Ubner et al., 2004). Comparing the CE profiles in the three types of samples, a broader third

peak (or stronger trailing) was observed in FA samples. The third peak was also closer to the second peak in the FA samples. Unlike HA and FA samples, the third peak in the unprocessed NOM sample was higher than the broad second peak.

Electropherograms of the three Nordic reservoir samples show the similar three-peak pattern (Fig. 1B). However, the second broad peak in the unprocessed Nordic NOM was flatter and lower. Leonardite and peat HA and FA show CE profiles (Fig. 1C) similar to those of the aquatic HA and FA samples. Three peaks appeared also in CE profiles of Elliott soil HA and FA (Fig. 1D). These peaks, however, were not as resolved as those of other samples. Rather than a round-top shape, the second peak of Elliott soil HA was asymmetric and relatively sharp. In the 13 electropherograms, the humic "hump" was centered more or less at the apex of the second peaks. However, the third peak was more like a surge on the trailing of the humic "hump." The third peak in FA samples was more like part of the "hump." This difference could be used to distinguish FA from HA by CE analysis.

Effects of Autoclaving on Capillary Electrophoresis Patterns

After 1-h autoclaving, all samples showed additional peaks in their electropherograms whereas the three original peaks remained (Fig. 2). Several minor peaks appeared before the first original sharp peak (i.e., the peak at 4.9 min in Fig. 2A). More significantly, a strong sharp peak (i.e., at 5.8 min in Fig. 2A) appeared between the first and second original peaks among all samples (Fig. 2). Among the three original peaks, the least conspicuous changes were observed in the first peak. The broad second peak generally became smaller in the autoclaved samples. The third original peak was flatter in some autoclaved samples (Fig. 2A) or even not apparent in some other samples (Fig. 2D). Therefore, we conclude that the additional CE peaks that appeared after autoclaving were the products of the heat decomposition and/or polymerization of the samples induced by autoclaving, mainly due to changes of the component represented by the broad second peak, with some contributions from the component of the third peak as well.

Fluorescence Characteristics of the IHSS Samples

A representative EEM fluorescence spectrum of the Nordic NOM is presented in Fig. 3. The visual "peak-picking" indicates that the Nordic NOM probably contained three fluorophores: the strongest and broad peak had an excitation maxima of <240 nm and emission maxima at ~440 nm, a moderate peak at an excitation of ~315 nm and emission at ~440 nm, and a weak peak at an excitation of ~305 nm and emission at ~350 nm. The PARAFAC modeling analysis fitted data and allowed the number of components to vary from two to eight. The modeling results indicated that the four-component model best fitted the 13 IHSS samples based on core consistency diagnostic criteria (Fig. 3). Component 1 had an excitation maximum at <250 nm and a broad emission maxima from 300 to 450 nm. In Component 2, the peak was in the upper left corner, at about 250 nm for excitation and 500 nm for emission. Components 3 and 4 both had two maxima peaks. However, the emission maxima were at 430 nm in Component 3, and at 470 nm in Component 4. The second excitation maximum was centered at 320 nm in Component 3. In Component 4, the second excitation maximum was wholly separate from the highest maximum, and centered at 360 nm. Previously, we reported a three-component model for six of

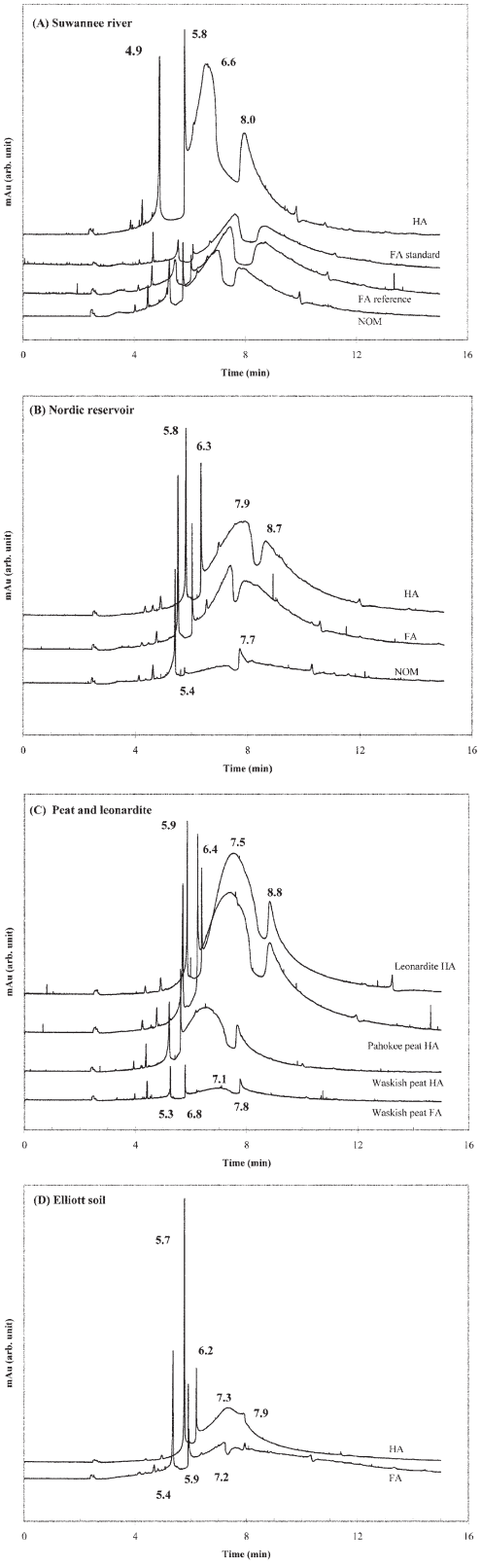

Fig. 2. Capillary electropherograms of the IHSS samples after autoclaving. HA, FA, and unprocessed NOM samples were from Suwannee river (A), Nordic reservoir (B), peat and leonardite sources (C), or Elliott soil (D).

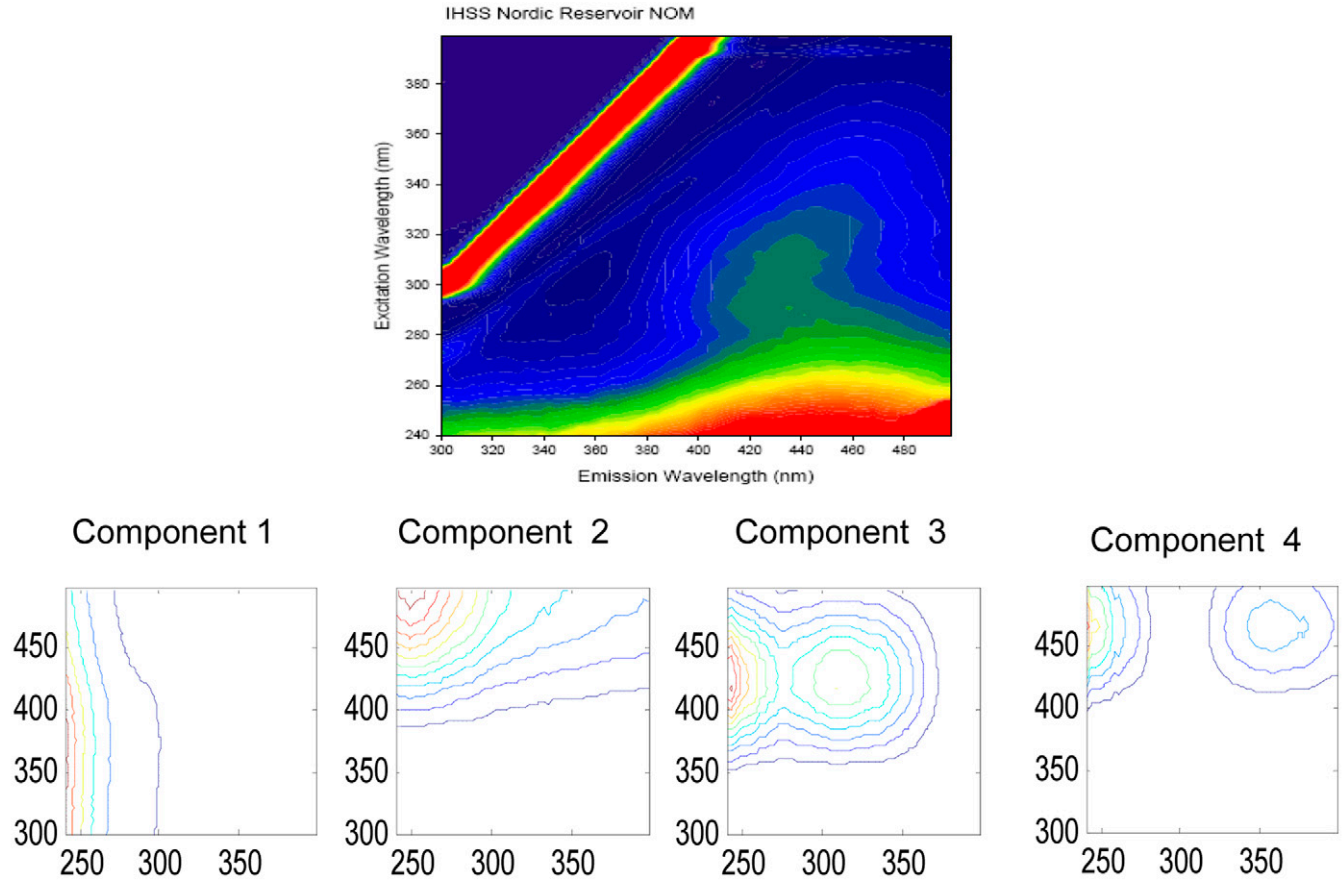

Fig. 3. Excitation-emission matrix spectrum of Nordic Reservoir NOM and the spectral characteristics of the four PARAFAC components.

these 13 IHSS samples (He et al., 2006). The Components 2 and 3 in this study were previously identified as Components 1 and 3, respectively (He et al., 2006; Ohno and Bro, 2006). The Component 2 identified in the six terrestrial IHSS samples was most similar to Component 1 in this work. However, a minor increase in emission absorbance near 500 nm in the previous Component 2 (Fig. 7 in Ohno and Bro, 2006) implies that a portion of current Component 4 was included in previous Component 2. Identification of the additional Component 4 in this work might be due to the larger data set, which included 26 untreated and autoclaved samples and 38 CE fractions.

Concentrations of the individual components for each sample were estimated in the first loading of the PARAFAC model, and the relative fractional distributions of the components for the 13 IHSS samples are shown in Fig. 4. The relative component distributions presented here are based on their relative contributions to the fluorescence signal, rather than on their true chemical concentrations. Expression of their distribution on a chemical concentration basis would require knowledge of the quantum fluorescence efficiencies of the individual components, which are unknown.

Component 1 generally was present in the greatest relative quantity across in all 13 IHSS samples (Fig. 4). Autoclaving lowered the content of Component 1 in all six HA samples; but increased the content in three of the five FA and in both unprocessed NOM samples. Based on Stedmon et al. (2003), Component 1 was the so-called "A" peak which is a strong UV humic-like peak. Thus, heat decomposition induced by autoclaving was able to break down some "humic-like" materials in these IHSS HAs.

The classification of Component 2 was unknown with regard to the fluorophore types that have been previously reported. Component 2 was less abundant than Component 1 in most samples, and autoclaving had less impact on Component 2, as the change after autoclaving was small. However, autoclaving decreased its content in all aquatic samples. This decrease indicated the lability of Component 2 in aquatic samples, which might be the cause of its low abundance as compared with the terrestrial samples.

Even though Component 3 was assumed to be a weak peak from "humic-like" materials of terrestrial sources (Stedmon et al., 2003), it was less abundant in the HAs than in FA and NOM samples. Autoclaving also had less impact on Component 3 than on other components.

Component 4 was the least abundant of the four components, and its identity is ambiguous at this time. Similar to Component 3, Component 4 was more abundant in the FAs and NOMs than HAs. The change induced by autoclaving was greater in Component 4. For the HA samples, autoclaving seemed to induce opposing changes in Component 1 and 4.

Distribution of Fluorophore Components in Capillary Electrophoresis Fractions

As CE separated the 13 samples to three peaks or fractions, and analysis of fluorescence spectroscopic data indicated that four fluorophore components were present in each sample, this led to the question whether any of the fluorophore compo-

nents are associated with a specific CE fraction. Therefore, we separated the three CE fractions from each of the 13 samples to analyze their fluorophore components. Because CE involves liquid samples on the scale of nanoliters, we extended the loading time from 5 s to 30 or 40 s. Due to this, the elution time and shape of CE peaks were not exactly identical to those in Fig. 1, however, the three-peak feature remained unchanged as shown by the representative electropherogram of Suwannee River HA (Fig. 5). The top 8 to 15% of each peak was collected. The CE separation was repeated 10 to 23 times to collect sufficient quantities of these fractions for fluorescence spectroscopic analysis (Table 2).

The four fluorophore components were present in all three CE fractions. However, the distributions of the four components differed among the three fractions (Fig. 6). In Fig. 6, the *x* axis is the relative abundance of the component in the original samples, and the *y* axis is the relative abundance of the component in the fractions. Any data point above the diagonal line indicates that the fluorophore component was enriched in that fraction compared with the original sample; any data point below the diagonal line indicates relative depletion of the fluorophore component in that fraction.

For Component 1 (Fig. 6A), all data points except one pair of Fraction 1 and 2 were above the diagonal line, indicating that the abundance of Component 1 in the two fractions was greater than in their original samples. The data points of Fraction 3 were relatively close to the diagonal line, indicating that the abundance of Component 1 in Fraction 3 approximated its abundance in the original samples. In contrast, the data points of Component 2 for Fraction 1 and 2 were below the diagonal line, indicating less abundance of Component 2 in these two fractions than in the original samples (Fig. 6B). Similar to Component 1, the data points of Fraction 3 were close to the diagonal line. Data points of Component 3 in Fraction 1 fluctuated considerably around the diagonal line (Fig. 6C). Data points for Fraction 2 were generally below the diagonal line; data points for Fraction 3 were close but generally below the line (Fig. 6C). For Component 4 (Fig. 6D), most of the data points were above the diagonal line, but the data points for Fraction 3 were closer to the line than were data points for Fraction 1 and 2. In summary, Fraction 1 was rich in Component 1 and 4, sparse in Component 2, but less changed for Component 3. Fraction 2 was rich in Component 1 and 4, but sparse in Component 2 and 3. The distribution of the four components in Fraction 3 was similar to that of the original samples.

DISCUSSION

The CE profiles of NOM are affected by many factors, such as different buffer systems, buffer concentrations, buffer pH, ionic strength, and sample concentration (Schmitt-Kopplin et al., 1998b; Egeberg and Bergli, 2002; Schmitt-Kopplin and Kettrup, 2003; Schmitt-Kopplin and Junkers, 2003; Peuravuori et al., 2004). We applied the analytic conditions reported by Pompe et al. (1996), and observed similar CE profiles of the Suwannee River HA and FA samples, which were reported in their study. Pompe et al. (1996) were not able to classify their peaks because no standards are available for the individual fractions. As the migration behavior of humic substances in CE depends on their charge-to-size ratio and the

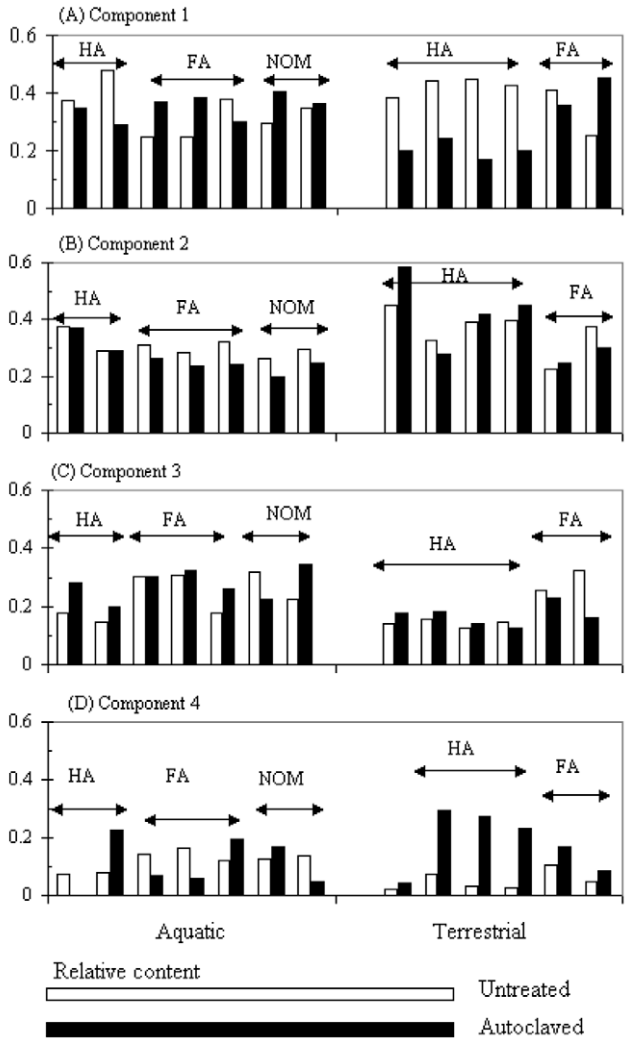

Fig. 4. Distribution of the four PARAFAC components among the 13 IHSS samples.

degree of ionization of their acidic phenolic and carboxylic group is governed by the CE buffer pH, some general interpretations could be made. The first peak which varied greatly in size with types of buffer, could be considered as the "neutral peak" produced by non-ionic components in the sample matrices (Garrison et al., 1995). The majority of humic substances or the humic "hump" materials were eluted in the second peak. There were marked differences in the relative proportions of

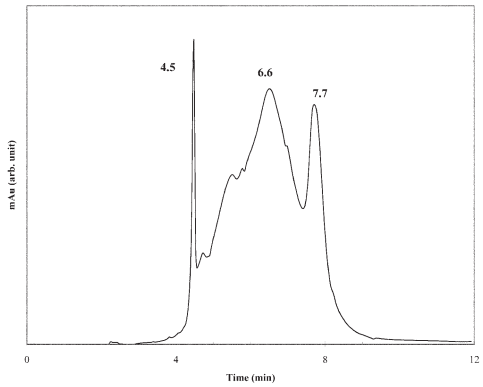

Fig. 5. Electropherogram of the Suwannee River HA used for the CE fraction collection.

the individual fractions and in the absorption intensities of the second peak of the HA and FA from the same origin, as observed previously (Pompe et al., 1996). The third sharp peak which appears in CE profiles of HA samples has been proposed to represent phenolic hydroxyl groups (Garrison et al., 1995) or HA-borate complexes formed through interaction of the sample with the borate buffer solution used in CE analysis (Schmitt-Kopplin et al., 1998b). The borate complexes were formed through phenolic functional group components in HA and FA that were identified by spiking the fulvic samples with model compounds and comparing the UV spectra (Schmitt-Kopplin et al., 1998a; Schmitt-Kopplin and Junkers, 2003). However, direct characterization of these peaks seems difficult if not impossible due to the limitation in sample loaded in CE analysis.

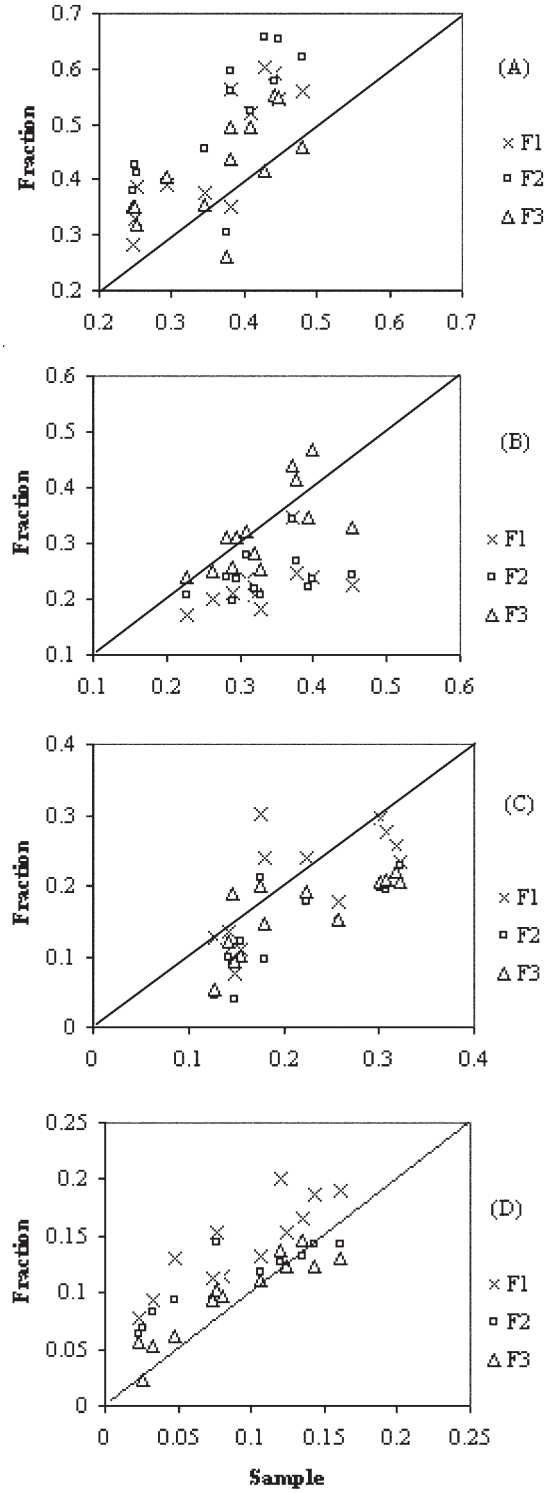

Fig. 6. Relationship of the relative abundance of fluorophore components in the original samples and the three CE fractions. (A), Component 1; (B), Component 2; (C), Component 3; (D), Component 4.

Fluorescence spectroscopy is a highly sensitive analytical method for characterizing organic matter. In this work, we established a general relationship or a trend between the three CE fractions and the four fluorophore components revealed by PARAFAC modeling analysis of EEM fluorescence data. The PARAFAC fluorophore components are not real molecules, but rather are mathematical constructs representing noninteracting ligands whose modeled parameters closely mimic the actual mixture of fluorescing compounds present in the samples (Ohno and Bro, 2006). Thus, these four fluorophore components in our work are better viewed as "quasi-particles" in the sense defined by Sposito and Blaser (1992), and they describe the average behavior of discrete, diverse classes of compounds present in the samples (Ohno and Bro, 2006). Their general nature could partly explain why all four components were present in the three CE fractions of each of the 13 IHSS samples. It has been previously stated that the separate CE peaks may not necessarily represent any distinct humic fractions, but may instead be artifacts caused by interactions of borate ions in the CE buffer solution and humic substances (Schmitt-Kopplin et al., 1998a; Schmitt-Kopplin et al., 1998b; Peuravuori et al., 2004). The different levels of enrichment or depletion of the components in the three CE fractions as shown by PARAFAC modeling suggest that these "quasi-particles" or groups of compounds in these humic samples were just at least partly separated by CE. Thus, CE peaks should not be considered as artifacts. For example, the first CE "neutral peak" was enriched in a strong "humic-like" fluorophore Component 1 and the undesignated Component 3. Autoclaving changed Component 1 and 4 in opposite manners, which suggested that these two components were differently impacted by a heating process.

In this work, we just collected CE fractions from unautoclaved samples. Autoclaving induced the appearance of additional CE peaks and changes in EEM fluorescence spectra. Correlation of the additional CE peaks with changes in the four components would certainly provide insight into the chemical structures of the IHSS samples. This is because EEM fluorescence and CE classify complicated organic components in NOM samples into general key groups (i.e., CE peaks and EEM components). This classification will allow us work on NOM with a much small "set" of key components and it enables us to generalize how "different" types of key organic matter components may be involved in important soil environmental processes.

CONCLUSIONS

In this study, we characterized 13 IHSS humic and NOM samples using CE and EEM fluorescence spectroscopy. The capillary electropherograms of the13 samples all showed three major peak features. The pattern and shapes of the peaks, however, were different among the samples. Especially, the "humic-hump" was centered more or less at the apex of the second

peaks in HAs. However, the "hump" resided in between the second and third peaks in FA samples. Excitation-emission matrix fluorescence spectroscopic analysis revealed that each of the 13 samples contained four fluorophore components, whose relative abundance varied among the three CE fractions. This implied that the "quasi-particles" or groups of compounds in these humic samples were separated at least partly by CE. Excitation-emission matrix fluorescence spectroscopy with PARAFAC modeling analysis of the CE peak fractions demonstrated that the peaks in the CE of the 13 IHSS samples were not due to artifacts, but represented chromatographic separation by the column. Autoclaving these samples for 1 h changed the CE profiles and portions of the four components, indicating that this combined approach can be used to investigate the dynamics of NOM changes. The combination of CE and EEM fluorescence spectroscopy together provided a novel approach for characterizing humic substances. Application of this approach to explore the characteristics of soil humic substance in relation to different cropping management practices and ecosystem functions is underway.

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